

## Differential gene expression in earthworms exposed to different concentrations of Cadmium

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**Introduction.** Cadmium (Cd) is a common contaminant found in many foods, accumulates throughout life and, in high doses, is a significant health hazard for humans. Cadmium is of interest because its concentration in agricultural soils has become elevated over time; with dietary intake in Western countries often close to tolerable limits. Analysis of change in gene expression in response to environmental stressors could potentially aid in risk assessment and biomarker development for human studies. *Eisenia fetida* are commonly used in ecotoxicological studies.

**Object.** To look at the impact of cadmium concentration on gene expression through comparative analysis of transcriptome data from *E. fetida* exposed to zero, low, medium and high doses of cadmium.

**Methods.** Artificial soil was spiked with three different Cd solutions; low (30mg/kg), medium (90mg/kg) and high concentration (270 mg/kg). Clean artificial soil was used for control. After a one week pre-incubation period at  $23 \pm 1^\circ\text{C}$  in the clean artificial soil, the fully clitellated adult worms were introduced to the Cd spiked soil (10 worms per beaker). The beakers were covered by fabric net and incubated under minimal light conditions. During the test period, the worms were fed oats once a week (5g per beaker) and water loss from evaporation was replenished every 2 days. After 28 days worms were removed from the test soil, and depurated. After depuration, the worms were washed with RNAase, DNase free water and a QIAGEN minikit was used to extract RNA prior to sequencing on an Illumina HiSeq 2500. A de novo transcriptome assembly of *E. fetida* was assembled using Trinity, Trimmomatic was used to quality trim reads. Trinotate was used to functionally annotate the transcriptome file and Transdecoder to generate peptide sequence files. Results from BLASTX, BLASTP and HMMER searches of the transcriptome file were loaded into a SQLite3 database. To identify differentially expressed transcripts; each of the original sequence files were aligned to the de novo assembled transcriptome using Bowtie and then RSEM was used to estimate expression values based on alignment. EdgeR was used to calculate differentially expressed genes between the four conditions.

**Results.** An annotated transcriptome was produced and differentially expressed genes analysed with a cut off of  $P=0.001$  and with a minimum of 15 fold change in gene expression level there were 50 genes identified as significantly differentially

expressed between the four conditions. Different concentrations of Cadmium caused significantly differentially expressed genes. The greatest change in gene expression occurred between the control and the maximum cadmium concentration.

	0	cd30	cd90	cd270
0	0	7	7	28
cd30	7	0	9	12
cd90	7	9	0	7
cd270	28	12	13	0

*Table 1. Comparative analysis of differential gene expression at different cadmium concentrations, number of genes identified with 15 fold change in gene expression at P=0.001 significance*

**Conclusion.** Comparative analysis of gene expression data can provide insight as to the molecular mechanisms that underlie and link environment and genome in terms of environmental exposure to toxins and the phenotypic outcome of health and recovery of the individual and the population.

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